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Spontaneous Neoplasms in Fishes. VII. A Spermatocytoma and Renal Melanoma in an African Lungfish, *Protopterus annectens* (Owen)

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(Plates I-IV; Text-figure 1)

Introduction

A LARGE tumor mass, identified as a spermatocytoma (Nigrelli & Jakowska, 1952), was found in a single African lungfish, *Protopterus annectens* (Owen), which died in the New York Aquarium. The growth involved the posterior part of the urogenital system. A detailed microscopic examination of various parts of the kidneys showed that a melanoma was also present. Insofar as is known, neither spermatocytoma nor renal melanoma has hitherto been reported in fish. This paper deals with the histology and cytology of these growths, together with a redescription of the normal urogenital system of *Protopterus annectens.*

Materials and Methods

The fish, which was 51 cm. long, had been in captivity for about five years. Autopsy showed that the tumor had caused a stenosis of the lower intestinal tract and cloaca. The affected urogenital system, together with a part of the large intestine, was resected and fixed in 10% formalin. Sections from various regions and levels were stained with hematoxylin-eosin, iron-alum-hematoxylin, Feulgen, Masson, Giemsa and with Toluidin blue.

Normal testes and kidneys from alcohol-preserved specimens of African lungfish, obtained from the American Museum of Natural History through the courtesy of Dr. C. M. Breder, Jr., were studied for comparison. Some of this material was washed and refixed for eight hours in 10% formalin, and the histological results were found to be satisfactory.

Observations

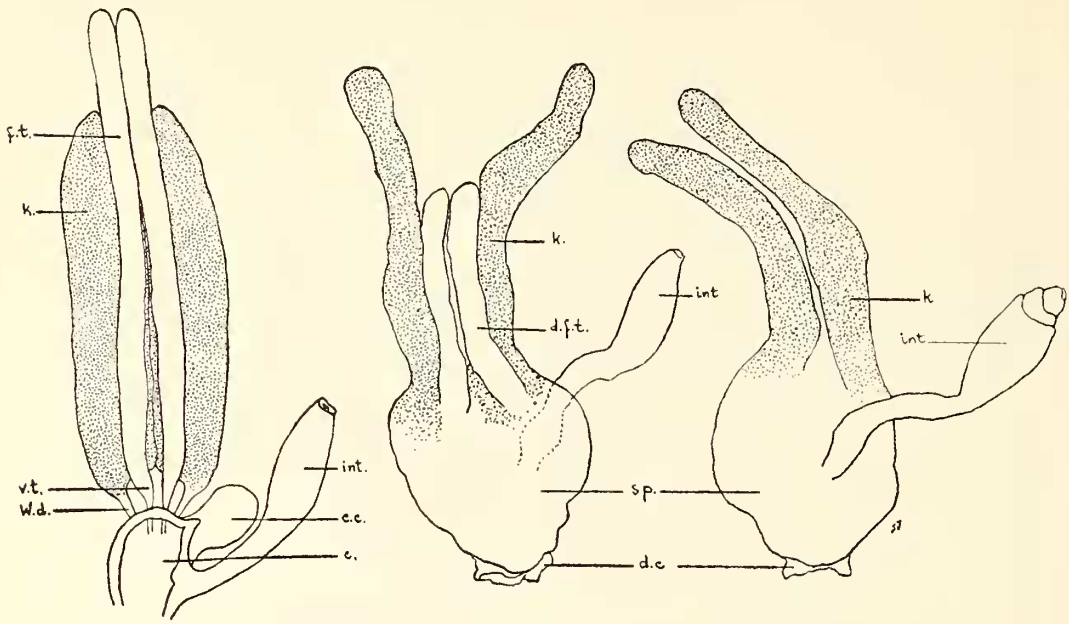
1. Normal Male Urogenital System

The present observations confirm the description given by Kerr (1901). The diagrammatic representation (Text-fig.1), in which the normal system (A) is compared with that of the tumorous fish (B & C), has been modified from Kerr's figures and description, with the omission of the vestigial Müllerian ducts.

The testes of the normal fish were paired and elongated. Each testis consisted of two parts: the anterior, sperm-producing or formative portion (Text-fig. 1A, f.t.) and a short vesicular testis (Text-fig. 1A, v.t.) which, according to Kerr's interpretation, serves as a duct and a *vesicula seminalis.* The kidneys were also elongated but usually shorter than the testes. They were fused in the posterior region but communicated with the cloaca (c.) through Wolffian ducts (W.d.). A blind dorsal diverticulum, the cloacal caecum (c.c.), was also present.

Microscopically, the normal testes showed characteristic interstitial elements and seminiferous tubules with cells in similar stages of spermatogenesis (Pl. I, Fig. 1). Spermatocytes in Metaphase I, with conspicuous bivalents, were relatively common. The lumen of some of the tubules contained mature sperms, each with a long pointed acrosome, an oval head (15 microns along the long axis), a round middle-piece (about 1 micron in diameter) and a tapering tail which was considerably longer than all the preceding parts.

The normal kidneys were covered by a thin layer of fibrous tissue in which fat cells and scattered melanophores occurred (Pl. I, Fig. 2).



TEXT-FIG. 1. A.—Diagrammatic representation of the normal male urogenital system in *Protopterus annectens* (Owen). Dorsal aspect. B.—Diagram of the urogenital system of the fish with spermatocytoma. Dorsal view. C.—Diagram of the same urogenital system as in B. Ventral view. Stippled areas represent renal tissue. Abbreviations: *c.* cloaca; *c.c.* cloacal caecum; *d.c.* degenerated cloacal region; *d.f.t.* degenerated formative testis; *f.t.* formative testis; *int.* large intestine; *k.* kidney; *sp.* spermatocytoma; *v.t.* vesicular testis; *W.d.* Wolffian duct.

Typical glomerular and tubular structures were present, and mitotically active hemopoietic regions were evident near the surface as islets of granulocytes and other blood elements surrounded by adipose tissue.

2. SPERMATOCYTOMA

The growth was conspicuous externally by a distention of the posterior part of the body in the vicinity of the pelvic fins. Internally, the tumor was found associated with the urogenital system and was localized in the posterior region at a level corresponding to the vesicular testes and where the kidneys are normally fused (Pl. II, Figs. 3, 4). The anterior parts of the kidneys and testes were not invaded by the growth. On the ventral side, it was found to involve the large intestine (Pl. II, Fig. 4); the cloaca was highly pigmented and showed evidence of pathological changes. The tumor was a bilaterally symmetrical ovoid, solid mass which measured 50 × 40 × 20 mm and was covered by a lightly pigmented capsule. When cut sagitally (Pl. II, Fig. 5), the growth appeared white and granular.

Microscopically, the distal portion of the testicular part of the tumor complex, which corresponds to the formative testes in the normal fish, lacked seminiferous tubules; instead, it consisted of an extremely thickened fibrous tube with papillary folds lined by a single layer of "germinal epithelium" (Pl. III, Fig. 6). The cells in the latter were large and round, with vesicular nuclei 12-22 microns in diameter which contained weakly-staining fine chromatin strands and one or two small but conspicuous nucleoli. These cells were identified as early spermatogonia. The fibrous connective tissue contained peculiar long, spiral-shaped nuclei (Pl. III, Fig. 7) and scattered melanophores, granulocytes and melanin-bearing macrophages. The presence of a few mature sperms in the region of the tumor which corresponds to the anterior part of the vesicular testes in the normal fish suggested that the shortened, fibrous distal part represented the formative testes, which had undergone considerable change in character.

The spermatocytoma occurred in the region where the posterior part of the vesicular testes is found. This tumor probably arose from vestigial elements of germinative nature. Since there was no evidence of vesicular testes and Wolffian ducts, it is assumed that these struc-

tures were invaded and completely obliterated by the proliferating tumor cells. The growth showed no cystic or teratomatous formations, no increased numbers of interstitial cells or concretions indicative of aging. The cellular elements consisted of numerous irregularly grouped, round primary spermatocytes and sparsely developed connective tissue (Pl. III, Fig. 8). No seminiferous tubules were present and degenerated cells were rare. Vascularization was restricted generally to the surface of the growth and in some regions there were groups of plasma cells, although no inflammatory reaction was evident.

The primary spermatocytes in the tumor tissue exhibited stages of first meiotic prophase, such as diplotene, and diakinesis with characteristic chiasmata (Pl. III, Fig. 9). Orientation of bivalents on the metaphase plate, anaphase separation without apparent lagging of chromosomes and completed telophase were also seen. Whenever it was possible to determine the number of chromosomes in Metaphase I or in Metaphase II, it corresponded closely to the number reported by Wickbom (1945) from normal material, namely n=17. In addition to the primary spermatocytes, other stages of spermatogenesis were occasionally found. Thus, cells identified as spermatogonia had nuclei characterized in the resting stage by the presence of heterochromatic bodies (prochromosomes), varying slightly in number, but relatively similar in size and shape. Two to four nucleoli were observed in these cells. The nuclear diameter of the spermatogonia was approximately 13-15 microns, as compared to the nuclear diameter of 10 microns for the typical primary spermatocytes and 7 to 8 microns for the secondary spermatocytes. In division, the spermatogonia exhibited the hollow spindle characteristic of this group of animals (Wickbom, 1945). It was assumed that meiosis in the spermatocytoma was successfully completed in some instances. Small round cells, with darkly staining nuclei 4 to 5 microns in diameter, found in groups of fours, were believed to be spermatids. Mature sperms, however, were not found in the tumor tissue.

3. Melanoma of the Kidney

The spermatocytoma grew around and into part of the posterior kidney with very little or no destruction of renal tissue. In other regions of the kidneys, however, there was an extensive invasion by capsular melanophores. In these areas, the capsule was especially hypertrophied, measuring about 2,000 microns in thickness, and contained tightly packed melanophores (Pl. IV, Fig. 10) which infiltrated the glomerular, tubular and hemopoietic regions of the kidney (Pl. IV, Fig. 11). Many of the tubular elements showed typical hyaline degeneration (Pl. IV, Fig. 12). Hemosiderin-like bodies of various sizes were seen in the cells of degenerating tubules and were also found as large scattered deposits in these regions. A tubular portion of the kidney, located outside the pigmented capsule, was apparently undamaged; it was supplied by several large blood vessels passing near the surface.

Discussion

There is considerable confusion in the literature concerning the nature of testicular tumors. Ewing (1940) was of the opinion that all such tumors are teratomatous in origin. Willis (1948), however, has presented evidence to show that testicular tumors in man and other mammals can be separated and classified as (1) seminomas or spermatocytomas, (2) teratomas, (3) carcinomas or adenomas of the excretory ducts and related Sertoli-cell tumors, and (4) interstitial-cell tumors. The confusion is due in part to the fact that seminomas may co-exist with teratomas which sometimes obliterate the structure of the seminomas, and, in part, to the fact that diagnostic cellular elements are often difficult to determine. It is only by careful cytological examination that one can recognize with any degree of certainty the cells of mammalian seminomas as spermatogonia or spermatocytes. No such difficulty is encountered in the testicular tumor of the lungfish, even though the typical seminiferous tubule arrangement is absent. Cytologically, the tumor presents a striking picture of normal spermatogenesis. The individual cells retain the characteristics of the species, such as the normal number of chromosomes, before and after reduction, the hollow spindle in the diploid cells (spermatogonia) and the typical heterochromatic bodies (prochromosomes) in the resting stage.

Summary

A spermatocytoma and a melanoma of the kidney in the African lungfish, *Protopterus annectens* (Owen), are described. This is apparently the first instance of such tumors reported for fish. The spermatocytoma had its origin in the posterior part of the vesicular testes and was composed chiefly of primary spermatocytes in various stages of first meiotic division. The growth involved the vesicular testes, Wolffian ducts, part of the large intestine and cloaca. The melanoma had its origin in the pigmented capsule of the kidney; it invaded and destroyed much of the renal parenchyma.

The urogenital system of the normal male *Protopterus annectens* is redescribed for comparison.

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EXPLANATION OF THE PLATES

Plate I

Fig. 1. Section of testis and kidney of a normal *Protopterus annectens*. The seminiferous tubule shows mature sperms in the lumen. Note also the granulocytopoietic portion of the kidney. Masson. 450 ×.

Fig. 2. Excretory part of the kidney with thin fibrous capsule and a few melanophores in the subcapsular region; fatty tissue around the kidney with granulocytic blood elements. Hematoxylin-eosin. 100 ×.

Plate II

Fig. 3. Dorsal view of the spermatocytoma of the urogenital system of *Protopterus annectens*. Note the relative size of kidneys and testes and compare with Text-figure 1A. Less than actual size.

Fig. 4. Ventral view of the spermatocytoma showing the relation to the large intestine. Less than actual size.

Fig. 5. Spermatocytoma cut sagitally. Thin pigmented connective tissue capsule surrounding the solid white granular tumor. No cystic or teratomatous formations are present. Less than actual size.

Plate III

Fig. 6. Section through part of the distal portion of the tumorous testes showing thickened fibrous walls with a single layer of "germinal epithelium." This part corresponds anatomically to the formative testis in the normal fish. Note, however, the lack of seminiferous tubules. Hematoxylin-eosin. 75 ×.

Fig. 7. Elongated spiral-shaped nuclei in the fibrous tissue shown in Figure 6. Hematoxylin-eosin. About 1000 ×.

Fig. 8. Characteristic microscopic view of the spermatocytoma. The majority of cells are primary spermatocytes in Prophase I. The typical arrangement into seminiferous tubules, however, is absent. Masson's stain. 1000 ×.

Fig. 9. A few cells from the spermatocytoma; one cell showing chiasma formation, others with heterochromatic bodies typical of the resting stage in this species. Masson's stain. 1800 ×.

Plate IV

Fig. 10. Thickened capsular area of the kidney showing extensive destruction of the parenchyma by invading melanophores. Masson's stain. 25 ×.

Fig. 11. Details showing invasion and destruction of the granulocytopoietic region of the kidney by capsular melanophores. 1000 ×.

Fig. 12. Tubular region of the kidney with melanoma showing hyaline degeneration of the tubular cells. Hematoxylin-eosin. 250 ×.